HORI3.001AUS PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Hahn et al.	Group Art Unit: Unk
Appl. No.	:	Unknown	
Filed	:	Herewith	
For	:	METHOD OF MEASURING CERULOPLASMIN CONCENTRATION IN A BLOOD SPOT, KIT AND METHOD OF DIAGNOSING WILSON'S DISEASE (amended)	
Examiner	:	Unknown	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to examination on the merits, please amend the above-captioned U.S. application as follows:

TITLE:

Please amend the title as follows:

METHOD OF MEASURING CERULOPLASMIN CONCENTRATION IN A BLOOD SPOT, KIT AND METHOD OF DIAGNOSING WILSON'S DISEASE

IN THE SPECIFICATION

On page 1 of the Specification, after the Title of the Invention and before the Background of the Invention, please insert ---This application claims priority of KR 10-2001-0017100, filed March 31, 2001.---

On page 6 of the present specification, please insert the following after line 26:

---Brief Description of the Drawings

These and other feature of this invention will now be described with reference to the drawings of preferred embodiments which are intended to illustrate and not to limit the invention.

Figure 1 shows 10μg of purified ceruloplasmin and the same volume of hybridoma supernatant reacted together at 37°C for 30 minutes, and electrophoresed using nonparametric polyacrylamide gel at 4°C. After the electrophoresis, the sodium acetate (pH 5.7) containing 1mg/ml of p-phenylenediamine dyed over the gel at 37°C for two hours to confirm the oxidase activity of ceruloplasmin, and was decolorized using 50% ethanol. Lane 1=purified ceruloplasmin; Lane 2=ceruloplasmin with a ceruloplasmin specific antibody.

Figure 2 shows a standard curve for ceruloplasmin concentration obtained by the method of Example 10.

Figure 3 shows a standard curve for ceruloplasmin concentration obtained by the method of Example 11.

Figure 4 shows ceruloplasmin concentration in 5 normal individuals and 12 Wilson's disease patients.

Detailed Description of the Preferred Embodiment---

IN THE CLAIMS

Please cancel claim 2.

Please amend claims 1, 3-6, 8, 10-17 and 21 as shown. A complete claim set is provided for convenience.

1. (Amended) A method of measuring a holoceruloplasmin concentration in a blood spot based on a standard curve obtained through an assay using a holoceruloplasmin-specific polyclonal antibody and a holoceruloplasmin-specific monoclonal antibody.

2. CANCEL

3. (Amended) The method of claim 1, wherein the blood spot is collected using a blood filter paper.

4. (Amended) The method of claim 1, wherein the ceruloplasmin-specific polyclonal antibody is manufactured from the serum that is obtained by a rabbit immunized with purified human ceruloplasmin containing the holoceruloplasmin.

5. (Amended) The method of claim 1, wherein the ceruloplasmin-specific monoclonal antibody is manufactured from a hybridoma cell line obtained through fusion of mouse spleen cells with myeloma cells, selection and cultivation of the fused spleen cells to produce a monoclonal antibody, and wherein the spleen cells were obtained by immunization of a mouse with purified ceruloplasmin containing holoceruloplasmin.

6. (Amended) The method of claim 22, wherein the absorbance standard curve according to the enzyme-linked immunosorbent assay is drawn out by applying the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with horseradish peroxidase, respectively to a standard blood spot and a control reference blood spot.

7. (Original) The method of claim 6, wherein the enzyme-linked immunosorbent assay for drawing out the absorbance standard curve is based on a sandwich method using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with horseradish peroxidase, respectively.

8. (Amended) The method of claim 23, wherein the fluorescence standard curve according to the dissociation-enhanced time-resolved fluoroimmunoassay is drawn out by applying the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with europium, respectively to a standard blood spot and a control reference blood spot.

9. (Original) The method of claim 8, wherein the dissociation-enhanced time-resolved fluoroimmunoassay for drawing out the fluorescence standard curve is based on a sandwich

ELISA method using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with europium, respectively.

10. (Amended) A method of measuring a holoceruloplasmin concentration in a blood spot according to an enzyme-linked immunosorbent assay, the method comprising the steps of:

manufacturing a ceruloplasmin-specific polyclonal antibody;

manufacturing a ceruloplasmin-specific monoclonal antibody;

conjugating horseradish peroxidase on the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody;

manufacturing a standard blood spot and a control reference blood spot by removing a ceruloplasmin from a blood sample and adding a purified ceruloplasmin solution containing a holoceruloplasmin at a constant concentration to the blood sample;

drawing out an absorbance standard curve based on the standard blood spot and the control reference blood spot using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody; and

measuring a holoceruloplasmin concentration in a blood spot of a patient using the standard curve through an enzyme-linked immunosorbent assay.

11. (Amended) A method of measuring a holoceruloplasmin concentration in a blood spot according to a dissociation-enhanced time-resolved fluoroimmunoassay, the method comprising the steps of:

manufacturing a ceruloplasmin-specific polyclonal antibody;

manufacturing a ceruloplasmin-specific monoclonal antibody;

conjugating with europium on the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody;

manufacturing a standard blood spot and a control reference blood spot by removing a ceruloplasmin from a blood sample and adding a purified ceruloplasmin solution containing a holoceruloplasmin at a constant concentration to the blood sample;

drawing out an fluorescence standard curve based on the standard blood spot and the control reference blood spot using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody; and

measuring a holoceruloplasmin concentration in a blood spot of a patient using the standard curve through a dissociation-enhanced time-resolved fluoroimmunoassay.

12. (Amended) The method of claim 10, wherein the patient has Wilson's disease.

13. (Amended) The method of claim 10, wherein the step of removing the ceruloplasmin in the blood is made using a phosphate-buffered saline.

14. (Amended) The method of claim 10, wherein the standard blood spot and the control reference blood spot are manufactured by adding a ceruloplasmin with a known concentration to the blood without the ceruloplasmin.

15. (Amended) The method of claim 14, wherein the known concentration of the ceruloplasmin has at least 3 different values.

16. (Amended) The method of claim 10, comprising a further step of screening an antibody for neutralizing an oxidase activity of the holoceruloplasmin at the step of manufacturing the ceruloplasmin-specific monoclonal antibody.

17. (Amended) The method of claim 10, comprising a further step of purifying the antibody after the step of manufacturing the ceruloplasmin-specific monoclonal antibody.

18. (Original) A Wilson's disease screening kit reagent, comprising a holoceruloplasmin-specific polyclonal antibody, a holoceruloplasmin-specific monoclonal antibody, a standard blood spot, and a control reference blood spot.

19. (Original) A Wilson's disease screening kit, being characterized of measuring a holoceruloplasmin concentration in a blood spot based on an absorbance standard curve obtained through an enzyme-linked immunosorbent assay using a holoceruloplasmin-specific polyclonal

antibody, a holoceruloplasmin-specific monoclonal antibody, a standard blood spot, and a control reference blood spot.

20. (Original) A Wilson's disease screening kit, being characterized of measuring a holoceruloplasmin concentration in a blood spot based on an fluorescence standard curve obtained through a dissociation-enhanced time-resolved fluoroimmunoassay using a holoceruloplasmin-specific polyclonal antibody, a holoceruloplasmin-specific monoclonal antibody, a standard blood spot, and a control reference blood spot.

21. (Amended) A method of diagnosing Wilson's disease using the screening kit of claim 19.

Please add the following claims:

22. (New) The method of claim 1, wherein the assay is an enzyme-linked immunosorbent assay and the concentration of holoceruloplasmin is based upon an absorbance standard curve.

23. (New) The method of claim 1, wherein the assay is dissociation-enhanced time-resolved fluoroimmunoassay and the concentration of holoceruloplasmin is based upon a fluorescence standard curve.

24. (New) The method of claim 11, wherein the patient has Wilson's disease.

25. (New) The method of claim 11, wherein the step of removing the ceruloplasmin in the blood is made using a phosphate-buffered saline.

26. (New) The method of claim 11, wherein the standard blood spot and the control reference blood spot are manufactured by adding a ceruloplasmin with a known concentration to the blood without the ceruloplasmin.

27. (New) The method of claim 26, wherein the known concentration of the ceruloplasmin has at least 3 different values.

28. (New) The method of claim 11, comprising a further step of screening an antibody for neutralizing an oxidase activity of the holoceruloplasmin at the step of manufacturing the ceruloplasmin-specific monoclonal antibody.

29. (New) The method of claim 11, comprising a further step of purifying the antibody after the step of manufacturing the ceruloplasmin-specific monoclonal antibody.

30. (New) A method of diagnosing Wilson's disease using the screening kit of claim 20.

REMARKS

The claims have been amended to remove multiple dependencies and correct non-idiomatic English. The specification has been amended to recite the priority application and to include a Brief Description of the Drawings. Support for the description of Figure 1 is found on page 8, lines 24-32 of the specification. Support for the description of Figure 2 is found on page 13, lines 10-12. Support for the description of Figure 3 is found on page 13, lines 16-19. Support for the description of Figure 4 is found on page 13, lines 20-25.

As a result of this preliminary amendment, Claims 22-30 have been added and Claims 1, 3-6, 8, 10-17 and 21 have been amended. Claim 2 has been cancelled. Accordingly, Claims 1 and 3-30 are presented for examination. No new matter is being added herewith.

The specific changes to the specification and the amended claims are shown on a separate set of pages attaches hereto and entitled **VERSION WITH MARKINGS TO SHOW CHANGES MADE**, which follows the signature page of this Amendment. On this set of pages, insertions are underlined and deletions are struck through.

Conclusion

Should there be any questions concerning this application, the Examiner is invited to contact the undersigned agent at the telephone number appearing below. Please charge any

additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Sept. 26, 2001

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Appl. No. : **Unknown**
Filed : **Herewith**

VERSION WITH MARKINGS TO SHOW CHANGES MADE

TITLE:

The title has been amended as shown:

METHOD OF MEASURING CERULOPLASMIN CONCENTRATION ~~WITH ENZYME LINKED IMMUNOSORBENT ASSAY OR DISSOCIATION ENHANCED TIME-RESOLVED FLUOROIMMUNOASSAY~~ IN A BLOOD SPOT, ~~AND WILSON'S DISEASE SCREENING~~ KIT AND METHOD OF DIAGNOSING WILSON'S DISEASE

IN THE CLAIMS:

The claims have been amended as shown:

1. (Amended) A method of measuring a holoceruloplasmin concentration in a blood spot based on ~~an absorbance~~a standard curve obtained through an ~~enzyme-linked immunosorbent~~ assay using a holoceruloplasmin-specific polyclonal antibody and a holoceruloplasmin-specific monoclonal antibody.

2. CANCEL

3. (Amended) The method of claim 1 ~~or claim 2~~, wherein the blood spot is collected using a blood filter paper.

4. (Amended) The method of claim 1 ~~or claim 2~~, wherein the ceruloplasmin-specific polyclonal antibody is manufactured from the serum that is obtained by a rabbit immunized ~~by the~~with purified human ceruloplasmin containing the holoceruloplasmin.

5. (Amended) The method of claim 1 ~~or claim 2~~, wherein the ceruloplasmin-specific monoclonal antibody is manufactured from a hybridoma cell line obtained through fusion of mouse spleen cells with myeloma cells, selection and cultivation of the fused spleen cells to produce ~~an~~ a monoclonal antibody, and wherein the spleen cells were obtained ~~from an immunized~~by immunization of a mouse with ~~the~~ purified ceruloplasmin containing ~~the~~ holoceruloplasmin.

6. (Amended) The method of claim ~~1~~22, wherein the absorbance standard curve according to the enzyme-linked immunosorbent assay is drawn out by applying the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with horseradish peroxidase, respectively to a standard blood spot and a control reference blood spot ~~manufactured~~.

7. (Original) The method of claim 6, wherein the enzyme-linked immunosorbent assay for drawing out the absorbance standard curve is based on a sandwich method using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with horseradish peroxidase, respectively.

8. (Amended) The method of claim ~~2~~23, wherein the fluorescence standard curve according to the dissociation-enhanced time-resolved fluoroimmunoassay is drawn out by applying the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with europium, respectively to a standard blood spot and a control reference blood spot ~~manufactured~~.

9. (Original) The method of claim 8, wherein the dissociation-enhanced time-resolved fluoroimmunoassay for drawing out the fluorescence standard curve is based on a sandwich ELISA method using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody conjugated with europium, respectively.

10. (Amended) A method of measuring a holoceruloplasmin concentration in a blood spot according to an enzyme-linked immunosorbent assay, the method comprising the steps of:

manufacturing a ceruloplasmin-specific polyclonal antibody;

manufacturing a ceruloplasmin-specific monoclonal antibody;

conjugating horseradish peroxidase on the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody;

manufacturing a standard blood spot and a control reference blood spot by removing a ceruloplasmin from a blood sample and adding a purified ceruloplasmin solution containing a holoceruloplasmin at a constant concentration to the blood sample;

drawing out an absorbance standard curve based on the standard blood spot and the control reference blood spot using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody; and

measuring a holoceruloplasmin concentration in a blood spot of a patient using the standard curve through an enzyme-linked immunosorbent assay.

11. (Amended) A method of measuring a holoceruloplasmin concentration in a blood spot according to a dissociation-enhanced time-resolved fluoroimmunoassay, the method comprising the steps of:

manufacturing a ceruloplasmin-specific polyclonal antibody;

manufacturing a ceruloplasmin-specific monoclonal antibody;

conjugating with europium on the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody;

manufacturing a standard blood spot and a control reference blood spot by removing a ceruloplasmin from a blood sample and adding a purified ceruloplasmin solution containing a holoceruloplasmin at a constant concentration to the blood sample;

drawing out an fluorescence standard curve based on the standard blood spot and the control reference blood spot using the ceruloplasmin-specific polyclonal antibody and the ceruloplasmin-specific monoclonal antibody; and

measuring a holoceruloplasmin concentration in a blood spot of a patient using the standard curve through a dissociation-enhanced time-resolved fluoroimmunoassay.

12. (Amended) The method of claim 10~~ or 11~~, wherein the patient has Wilson's disease.

13. (Amended) The method of claim 10~~ or 11~~, wherein the step of removing the ceruloplasmin in the blood is made using a phosphate-buffered saline.

14. (Amended) The method of claim 10~~ or 11~~, wherein the standard blood spot and the control reference blood spot are manufactured by adding a ceruloplasmin with a known concentration to the blood without the ceruloplasmin.

15. (Amended) The method of claim ~~10 or 11~~14, wherein the known concentration of the ceruloplasmin has at least 3 different values.

16. (Amended) The method of claim 10~~ or 11~~, comprising a further step of screening an antibody for neutralizing an oxidase activity of the holoceruloplasmin at the step of manufacturing the ceruloplasmin-specific monoclonal antibody.

17. (Amended) The method of claim 10~~ or 11~~, comprising a further step of purifying the antibody after the step of manufacturing the ceruloplasmin-specific monoclonal antibody.

18. (Original) A Wilson's disease screening kit reagent, comprising a holoceruloplasmin-specific polyclonal antibody, a holoceruloplasmin-specific monoclonal antibody, a standard blood spot, and a control reference blood spot.

19. (Original) A Wilson's disease screening kit, being characterized of measuring a holoceruloplasmin concentration in a blood spot based on an absorbance standard curve obtained through an enzyme-linked immunosorbent assay using a holoceruloplasmin-specific polyclonal antibody, a holoceruloplasmin-specific monoclonal antibody, a standard blood spot, and a control reference blood spot.

20. (Original) A Wilson's disease screening kit, being characterized of measuring a holoceruloplasmin concentration in a blood spot based on an fluorescence standard curve obtained through a dissociation-enhanced time-resolved fluoroimmunoassay using a holoceruloplasmin-specific polyclonal antibody, a holoceruloplasmin-specific monoclonal antibody, a standard blood spot, and a control reference blood spot.

21. (Amended) A method of diagnosing Wilson's disease using the screening kit of claim 19~~ or 20~~.